



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 49/02, C07J 9/00	A1	(11) International Publication Number: WO 92/14493 (43) International Publication Date: 3 September 1992 (03.09.92)
(21) International Application Number: PCT/US92/01072 (22) International Filing Date: 20 February 1992 (20.02.92) (30) Priority data: 567,772 20 February 1991 (20.02.91) US (71)(72) Applicant and Inventor: CAPELLI, Christopher [US/US]; 4500 7th Street, Kenosha, WI 53142 (US). (72) Inventor: PAAREN, Herbert ; 415 N. Few Street, Madison, WI 53703 (US). (74) Agents: BENT, Stephen, A. et al.; Foley & Lardner, 1800 Diagonal Road, Alexandria, VA 22313 (US).		(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CI (OAPI patent), CM (OAPI patent), CS, DE, DE (European patent), DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GN (OAPI patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC (European patent), MG, ML (OAPI patent), MN, MR (OAPI patent), MW, NL, NL (European patent), NO, PL, RO, RU, SD, SE, SE (European patent), SN (OAPI patent), TD (OAPI patent), TG (OAPI patent). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: NON-PROTEIN INTRACELLULAR RECEPTOR BINDING CONJUGATES AND A METHOD OF USE THEREOF (57) Abstract A non-protein intracellular receptor binding molecule conjugated to therapeutic and diagnostic agents via a linker molecule is provided. A method for the intracellular delivery <i>in vivo</i> of therapeutic or diagnostic agents is also provided.		

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NON-PROTEIN INTRACELLULAR RECEPTOR BINDING
CONJUGATES AND A METHOD OF USE THEREOF

Field of the Invention

5 This invention relates to non-protein intracellular
receptor binding molecules (hereinafter referred to as
"receptor binding molecules") conjugated to therapeutic,
or to diagnostic agents via a linker molecule and a
method for the *in vivo* intracellular delivery of such
10 agents.

Background of the Invention

A number of targeting agents have been utilized with
varying degrees of success in the *in vivo* delivery of
diagnostic and/or therapeutic agents to target sites.
15 Antibodies, certain hormones, such as insulin, and other
proteins are exemplary of such targeting agents.

In many instances, particularly if the size of the
targeting agent is small relative to that of the
therapeutic or diagnostic agent, the preferred method of
20 conjugation is via a linker moiety. A number of such
linker moieties have been reported for use with
antibodies. For example, therapeutic agents conjugated
to antibodies via certain peptide linking molecules which
are susceptible to cleavage by enzymes are disclosed by
25 Goers et al. in U.S. patent No. 4,876,973. Goers also
discloses linker moieties comprised of non-cleavable
molecules such as amino acids, peptides and other organic
compounds. In addition, metal-ions conjugated to
antibodies via chelating compounds are reported in U.S.
30 patent No. 4,741,900.

The use of proteins and hormones as conjugated
targeting agents has been reported less frequently. In
this regard, Pozansky, in U.S. patent No. 4,749,570,
discloses the use of an insulin conjugate. In

particular, Pozansky discloses a complex comprising insulin, a protein, conjugated to an enzyme, the therapeutic agent, via albumin, the linker molecule. This complex is used to deliver the enzyme to the surface of the targeted cell.

In an effort to maximize the action of therapeutic agents, Wilbur et al. (European patent application 0 336 364) utilized a modified cellular substrate as a linking group to attach a ligand, such as a drug or radionuclide, to a targeting antibody or antibody fragment. The targeting antibody brings the modified cellular substrate linker into close contact with the targeted cell membrane. The linker is then transported across the cell membrane. Once inside the cell, the modified linker cannot be readily metabolized. Thus, the time the substrate is retained in the cell is greatly extended and the amount of time the cell is exposed to the therapeutic or diagnostic agent bound to the linker is concomitantly increased.

In general, targeting agents are large molecular weight moieties that share the common characteristic of specifically binding epitopes located on the cell surface. The most commonly employed targeting agents are antibodies and antibody fragments which are large molecular weight molecules which specifically bind epitopes located on the cell surface. The size of such targeting agents alone prevents their transport across the cell membrane.

Because of these properties, the usefulness of these targeting agents in bringing the therapeutic or diagnostic agent into close proximity with the targeted cell is limited. As a result, the effect of the therapeutic or diagnostic agent on the intracellular region of the targeted cell is minimized. A targeting agent that binds intracellular receptors is transported across the cell membrane. Therapeutic or diagnostic agents conjugated to such a receptor binding molecule may also be transported into the intracellular region of the

targeted cell where they are likely to be more effective.

An example of a non-protein steroid hormone intracellular receptor binding molecule used as a targeting agent is disclosed in U.S. patent

5 No. 4,882,141, wherein Baranczuk describes the use of 16-¹²³I-17- β -estradiol as a labeled targeting agent in certain imaging and therapeutic methods. These methods are specifically used for the treatment or imaging of tissue which contain steroid receptors.

10 Although β -estradiol is a relatively small molecule with an approximate molecular weight of 350, it can be labeled with ¹²³I because ¹²³I readily substitutes for a hydrogen atom on the estradiol molecule. Due to the small size of such non-protein intracellular receptor
15 binding molecules, direct conjugation of therapeutic or diagnostic agents other than a radionuclide such as ¹²³I is not feasible. Therefore, a need continues to exist for a means of conjugating therapeutic and diagnostic agents to intracellular receptor binding molecules so
20 that these receptor binding molecules can be more effectively exploited as targeting agents for the *in vivo* intracellular delivery of therapeutic and diagnostic agents.

Summary of the Invention

25 It is therefore an object of the present invention to provide a linker moiety which can be used to conjugate non-protein intracellular receptor binding molecules to therapeutic or diagnostic agents.

It is a further object of the present invention to
30 provide a method for the intracellular delivery *in vivo* of a therapeutic or diagnostic agent.

In accomplishing the foregoing objects, there has been provided, in accordance with one aspect of the present invention, a targeting agent for the
35 intracellular delivery of a therapeutic or diagnostic agent, comprising a conjugate comprised of (i) a non-protein molecule which binds an intracellular receptor,

(ii) a therapeutic or diagnostic agent and (iii) a linker moiety joining said agent to said non-protein molecule. In a preferred embodiment, the linker molecule is any functional organic compound or reagent with a functionality greater than 1 that is capable of covalently attaching to both the non-protein molecule which binds to an intracellular receptor and the therapeutic or diagnostic agent. In another preferred embodiment, the therapeutic agent is selected from the group consisting of pharmaceutical agents, enzymes, antibiotics, antimetabolites, antiproliferative agents, neurotransmitters, DNA radio-opaque dyes, radioactive isotopes, fluorogenic compounds, marker compounds, lectins, cell membrane altering compounds, photochemicals and boron-containing agents. In a yet another preferred embodiment, the diagnostic agent is selected from the group consisting of chelated radiopharmaceuticals, paramagnetic metals, and photodynamic agents.

In accordance with another aspect of the invention, a pharmaceutical composition is provided that is suitable for *in vivo* administration, comprising an effective amount of a conjugate comprised of (i) a non-protein molecule which binds an intracellular receptor, (ii) a therapeutic or diagnostic agent and (iii) a linker moiety joining said agent to said non-protein molecule and a pharmaceutically acceptable carrier.

In accordance with yet another aspect of the present invention, a use is provided of a conjugate comprised of aforementioned components (i), (ii) and (iii) in the preparation of an agent for use in a method for intracellular delivery *in vivo* of a therapeutic or diagnostic agent.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention,

are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

5 Detailed Description of the Preferred Embodiments

It has been discovered that conjugated non-protein intracellular receptor binding molecules can be effectively employed as targeting agents for the *in vivo* delivery of a number of therapeutic and diagnostic agents. It is the specificity of such a receptor binding molecule that renders it ideally suitable for the delivery of these agents to specific cells, tissues, organs or any other site with the particular receptor.

10 Intracellular receptor binding molecules are relatively small molecules that possess distinct structural characteristics which are critical to their ability to bind to a receptor. Conjugation of a therapeutic or diagnostic agent to such a molecule causes substantial interference with the structural features which determine the molecule's ability to bind a receptor. Thus, these molecules were not previously considered to be useful as targeting agents for the *in vivo* delivery of therapeutic and diagnostic agents. In accordance with the present invention, it has been determined that structural binding incompatibilities of this nature can be avoided by conjugating therapeutic or diagnostic agents to a receptor binding molecule via a suitable linker molecule.

Any intracellular receptor binding molecule that can be conjugated to a therapeutic or diagnostic agent via a linker compound which retains its ability to bind to the receptor is suitable for use within the present invention. Exemplary of suitable intracellular receptor binding molecules are adrenocorticosteroids, progestins, anti-progestins, estrogens, anti-estrogens (steroidal or non-steroidal), androgens and anti-androgens, as well as thyroid hormone compounds, vitamin D compounds and their

respective analogs. In a preferred embodiment, the intracellular receptor binding molecule is comprised of an analog of estradiol.

A linker molecule within the present invention binds
5 to the receptor binding molecule without compromising the critical structural characteristics which permit the conjugated molecule to be bound by the receptor. Moreover, a suitable linker molecule can bind to a number
10 of therapeutic and diagnostic agents without interfering with the activity of the agent. Linker molecules suitable for use within the present invention are comprised of at least two functional groups which bind with the non-protein targeting agent and the diagnostic or therapeutic agent. Linker molecules comprised of more
15 than one functional group are described herein as possessing a "functionality greater than 1." In accordance with the present invention, a linker molecule may include any compatible organic or inorganic compound which does not adversely affect the binding capacity of
20 the receptor binding molecule. Suitable linker molecules include but are not limited to organic and inorganic compounds with a functionality greater than 1 such as *ethylene diamine and diisocyanates, synthetic polymers such as polyethers, polyethyleneamines and polyamides;
25 biologic molecules and biologic molecule analogs such as peptides, monosaccharides and fatty acids; and biopolymers, for example polypeptides such as poly-L-lysine, poly-L-lysyl-DL-alanine, and polysaccharides and polysaccharide analogs, for example carbocyclic analogs
30 of sugar such as ribose. In a particularly preferred embodiment, the linker molecule is comprised of ethylene diamine conjugated to an acetylenic organic acid spacer of 7 atoms in length.

In accordance with the present invention,
35 intracellular receptor binding molecules can be conjugated to any therapeutic or diagnostic agent which retains its essential properties after attachment to the receptor binding molecule via the linker molecule. The

term "therapeutic agent" includes therapeutic agents which have been chemically modified or any other derivative forms of such agents which substantially retain their biological activity. Exemplary of such therapeutic agents are pharmaceuticals, toxins, fragments of toxins, alkylating agents, enzymes, antimicrobials, antimetabolites, antiproliferative agents, neurotransmitters, DNA, radio-opaque dyes, radionuclides, fluorogenic compounds, marker compounds, lectins, comgenic compounds which alter cell membrane permeability, photochemical compounds and boron-containing compounds for use in boron neutron capture therapy.

Antimicrobials suitable for use within the present invention include, are not limited to, streptomycin, neomycin, kanamycin, gentamicin, amikacin, tobramycin, streptomycin B, spectinomycin, ampicillin, sulfanilamide, polymyxin, chloramphenicol, acyclovir, vira A, symmetrel, nystatin and tylosine. Examples of suitable antineoplastics within the present invention include, but are not limited to, adriamycin, cerubidine, bleomycin, alkeran, velban, oncovin, fluorouracil, methotrexate, thiotepa, bisantrene, novatrone, thioguanin, procarabazine and cytarabine.

Pharmaceutical compositions comprising such agents conjugated to a receptor binding molecule via a linker molecule in a suitable carrier, including serum or physiological saline, with or without another protein such as human serum albumin. Dosage may be readily determined by a skilled artisan and may differ depending on the nature of the cellular disorder and the agents employed.

Photodynamic agents suitable for use with the present invention include, but are not limited to, porphyrins and modified porphyrins such as hematoporphyrin, hematoporphyrin dihydrazide, deuteroporphyrin dihydrazide and protoporphyrin dihydrazide, rose bengal, acridines, thiazines,

xanthenes, anthraquinones, azines, flavin and nonmetal-containing porphyrins, porphyrin-like compounds, methylene blue, eosin, psoralin and the like. Photosensitizers such as tetracyclines, sulfonamides, griseofulvin, phinothiazines, thiazides and sulfonylurea may be conjugated to receptor binding molecules pursuant to the present invention. Such photochemicals may be modified or synthetically prepared to absorb light at specific wavelengths. In addition, photothermolytic agents, such as Azure A, that can be activated at the targeted site by a light source, are suitable for use with the present invention.

In vivo administration of photodynamic agents conjugated to receptor binding molecules may involve the use of pharmaceutical compositions comprising the conjugate and any suitable carrier, including serum or physiological saline, with or without another protein such as human serum albumin. Dosage of pharmaceutical compositions according to the present invention may be readily determined by one of ordinary skill and will differ depending upon the nature of the cellular disorder and the agents used. The route of administration may be parenteral, with intravenous injection generally preferred.

Photosensitive agents may be activated by a light source which causes the reduction of singlet oxygen resulting in a toxic cellular effect. The specificity of the reaction can be maintained by selection of proper wavelength and photosensitive agent. The photosensitive agent can be activated at the targeted site with a laser or other light source via optical fibers or any other appropriate method.

In a similar vein, metal ions chosen for their cell killing properties can be conjugated to receptor binding molecules according to the present invention. Examples of suitable beta-emitting ions for therapeutic uses include, but are not limited to, ^{46}Sc , ^{47}Sc , ^{48}Sc , ^{76}Ga and ^{73}Ga , while ^{211}Bi , ^{213}Bi and ^{214}Bi are exemplary of suitable

alpha emitters within the present invention. In a preferred embodiment, an alpha-emitting metal ion comprised of ^{212}Bi is conjugated via a suitable linker molecule to a non-protein intracellular receptor molecule. Pharmaceutical compositions comprising a metal ion conjugated via a linker molecule to a receptor binding molecule and a suitable carrier can be administered parenterally. The preferred route of administration is intravenous and the proper dosage can be determined without undue experimentation by one skilled in the art.

Radionuclides suitable for therapeutic and diagnostic imaging according to the present invention include, but are not limited to, ^{90}Y , ^{67}Cu , ^{67}Ga , ^{68}Ga , ^{89}Zr , $^{99\text{m}}\text{Tc}$, ^{111}In , ^{123}I , ^{125}I , ^{131}I , ^{75}Br , ^{76}Br , ^{77}Br , ^{198}Au , ^{199}Au , ^{18}F , ^{105}Rh , ^{186}Re , ^{188}Re , ^{211}At , ^{203}Pb and ^{212}Pb . Examples of non-radioactive paramagnetic metals such as ^{157}Gd , ^{54}Fe , ^{56}Fe , ^{57}Fe , ^{58}Fe and ^{55}Mn which can be detected by nuclear magnetic resonance spectroscopy are suitable for tissue imaging according to the present invention. Certain lanthanides including praseodymium, neodymium, samarium, europium, gadolinium, terbium, dysprosium, holmium, erbium, thulium and ytterbium are useful in fluorescent diagnostic assays within the present invention.

In accordance with the present invention, radionuclides and metal ions may be conjugated to receptor binding molecules via a linker molecule and a chelating compound. Compatible chelators capable of coordinated bonding with a metal ion are utilized to attach the metal ions or radionuclides to the linker molecule. As used herein, the term "compatible chelator" means any compound that is able to donate electrons and combine to coordinate bonding with a metal ion to form structures called chelates or chelation complexes and is suitable for attachment to a receptor binding molecule via a linker molecule without loss of ability to chelate metal ions or radionuclides or loss of binding activity or specificity of the receptor binding molecule.

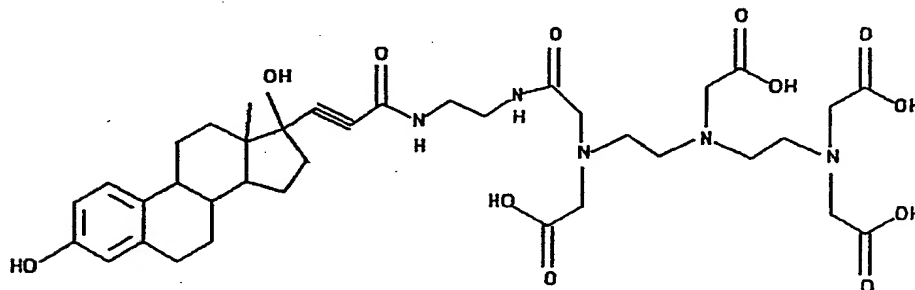
10

Exemplary of suitable chelators are diethylene-triamine-pentaacetic acid (DPTA), ethylene-diamine-tetraacetic acid (EDTA), desferroxamine, dimercaptosuccinic acid, 2,3-dimercaptopropane-sulfonic acid, metallothioein and cryptates, such as those described by Gansow et al. in *J. Heterocyclic Chem.* 18: 297 (1981).

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following examples are therefore, to be construed as merely illustrative and not limitative of the remainder of the disclosure in any way whatsoever.

Example 1: Preparation of a Conjugated Targeting Agent for NMR Imaging.

An estradiol/DPTA conjugate, capable of chelating the paramagnetic ion gadolinium (Gd) for use in NMR imaging, was synthesized. The structure of the estradiol/DPTA conjugate is presented below:



A 4X molar excess of methyl-lithium was added to a solution of 9.84 grams of 17- α -ethynylestradiol in 396 ml of anhydrous tetrahydrofuran (THF). Carbon dioxide was bubbled through the mixture at a low rate while being stirred for 3 hours at room temperature. The solution was then stirred at room temperature overnight. The reaction

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mixture was poured over ice and the resulting solution was acidified to a pH less than 3 with 2N sulfuric acid. The resulting intermediate product and the 17- α -ethynylestradiol starting material were then extracted with 300 ml ethyl acetate. One hundred and fifty milliliters of water was added to this solution and the pH was increased by adding a 5% sodium bicarbonate solution. The water layer containing the intermediate product was then separated from the ethyl acetate layer and the intermediate product precipitated by acidifying the solution with 2N sulfuric acid. The precipitate was extracted with ethylacetate and dried over magnesium sulfate.

After evaporating off the ethyl acetate, 3.58 grams of the dried intermediate product was dissolved in 140 ml of anhydrous THF containing 2.5X molar excess of N-hydroxysuccinimide and 1.1X molar excess of 1,3-dicyclohexylcarbodiimide and was stirred at room temperature for 4 hours. The reaction mixture was filtered and the filtrate was slowly dripped into a 50 ml THF solution containing a 3X molar excess of ethylene diamine and stirred overnight at room temperature. The solution was then filtered through a coarse paper filter.

After evaporation of the solvent, 100 mg of the intermediate product was dissolved in 1 ml of anhydrous dimethylformamide (DMF). The resulting solution was added to 2 ml DMF containing a 2X molar excess of diethylenetriaminepentaacetic dianhydride (DTPA) and then stirred for one hour at room temperature. Fifteen milliliters of a solution comprised of 0.5% sodium bicarbonate in water was added and stirred for 4 hours at room temperature. The estradiol/DTPA conjugate was precipitated by adding 2N sulfuric acid, filtered using a Pyrex® buchner funnel with a 0.9-1.4 μ fritted disc and dried under high vacuum overnight. The estradiol/DTPA conjugate was characterized using FAB mass spectrum analysis which yielded a molecular ion $M+H^+$ of 758 which

corresponded to the expected molecular weight for the derivative.

Example 2: Conjugation of Diagnostic Imaging Agent to Estradiol/Linker Molecule

5 The estradiol/DTPA conjugate was prepared according to Example 1. A 10 mM solution of the conjugate dissolved in water was prepared and the pH of the solution was adjusted to 7.0 using 0.1 N sodium hydroxide. To this solution, an equimolar amount of
10 gadolinium (Gd) chloride was added. The mixture was stirred for 1 hour at room temperature.

Example 3: In vivo NMR Imaging Using Conjugated Targeting Agent

15 Animal studies were performed to evaluate the effectiveness of an estradiol/gadolinium conjugate prepared according to Example 2 in targeting tissues rich in estrogen receptors. A test group consisting of 6 healthy female mice, each weighing 20-25 grams, were injected with an estradiol/gadolinium conjugate prepared
20 according to Example 2. Prior to administration of the conjugate, the mice were anesthetized using ketamine chloride at a dosage of 150 mg per kilogram body weight. The conjugate was injected intravenously in the tail twice daily for three days at a dosage of 1 μ M per
25 kilogram body weight. Three hours after the last infusion of conjugate, the test mice were euthanized and NMR studies were performed. An MR scan was performed utilizing a 9.4/8.9 cm (400MHz) Bruker AM-400 wide-bore multinuclear spectrometer. One to eight 1 mm cross-
30 sectional MR images of the pelvic region were taken of each animal. The relative contrast of the uterine tissue was determined by comparing the signal intensity of the surrounding tissue to the signal intensity of the uterine tissue. MR scans were performed on a control group of 8
35 healthy female mice of the same age and weight as the test group. The scan data obtained from the control

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group provided the baseline of the relative contrast between normal uterine tissue and surrounding tissue. The results are presented in Table 1.

TABLE 1

<u>Animal #</u>	<u>Contrast (Uterus/Surrounding Tissue)</u>
Test 1	++
Test 2	+
Test 3	++
Test 4	+++
Test 5	+++
Test 6	++
Control 1	--
Control 2	--
Control 3	--
Control 4	--
Control 5	--
Control 6	--
Control 7	--
Control 8	--

+ = Signal intensity ratio uterine/surrounding tissue greater than 1:1
- = Signal intensity ratio uterine/surrounding tissue equal to or less than 1:1

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In the test mice, the signal intensity of the uterine tissue was greater than that of the surrounding tissue. In the control group, the signal intensity of the uterine tissue was approximately equal to that of the surrounding tissue. The results illustrate that the estradiol/gadolinium conjugate of the present invention provided specific contrast in tissues containing estrogen receptors.

What Is Claimed Is:

1. A targeting agent for the intracellular delivery of a therapeutic or diagnostic agent, comprising a conjugate comprised of (i) a non-protein molecule which
5 binds an intracellular hormone receptor, (ii) a therapeutic or diagnostic agent and (iii) a linker moiety joining said agent to said non-protein molecule.
2. A pharmaceutical composition suitable for *in vivo* administration, comprising an effective amount of a
10 conjugate according to Claim 1 and a pharmaceutically acceptable carrier therefor.
3. A targeting agent according to Claim 1, wherein said non-protein molecule is selected from the group consisting of a adrenocorticosteroid, a progestin, and
15 anti-progestin, an estrogen, an anti-estrogen, an androgen, an anti-androgen, a thyroid hormone compound and a vitamin D compound.
4. A targeting agent according to Claim 1, wherein said linker molecule is comprised of at least two
20 functional groups capable of joining said therapeutic or diagnostic agent and said targeting agent and is selected from the group consisting of inorganic and organic compounds, biologic molecules, synthetic polymers and biopolymers.
- * 5. A targeting agent according to Claim 1, wherein said therapeutic agent is selected from the group consisting of pharmaceutical agents, enzymes, antibiotics, antimetabolites, antiproliferative agents, neurotransmitters, DNA radio-opaque dyes, radioactive
25 isotopes, fluorogenic compounds, marker compounds, lectins, cell membrane altering comgenic compounds, photochemicals and boron-containing compounds.
30

6. A targeting agent according to Claim 1, wherein said diagnostic agent is selected from the group consisting of chelated radiopharmaceuticals, paramagnetic metals, and photodynamic agents.

5 7. A method for the intracellular delivery *in vivo* of a therapeutic or diagnostic agent, comprising the steps of

(a) providing a pharmaceutical composition according to Claim 2; and

10 (b) administering said composition to a subject such that said therapeutic or diagnostic agent is taken up by target cells containing an intracellular receptor bound by said non-protein molecule.

15 8. Use of a conjugate comprised of (i) a non-protein molecule which binds an intracellular receptor, (ii) a therapeutic or diagnostic agent and (iii) a linker moiety joining said agent to said non-protein molecule and a pharmaceutically acceptable carrier, in the preparation of an agent for use in a method for intracellular
20 delivery *in vivo* of the therapeutic or diagnostic agent.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US92/01072

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC Int. cl(5): A61K 49/02, C07J 9/00, US. CL. 424/1.1		
II. FIELDS SEARCHED		
Minimum Documentation Searched †		
Classification System	Classification Symbols	
U.S.	424/1.1, 424/9, 260/397.2, 514/169	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ‡		
III. DOCUMENTS CONSIDERED TO BE RELEVANT †		
Category *	Citation of Document, † with indication, where appropriate, of the relevant passages ‡	Relevant to Claim No. ‡
X	US, A, 4,215,045 Published 29 July 1980, (Knapp, Jr.) (Claims 11-15, 16-19)	1-5,1-4,6,7
X	US, A, 4,938,897 Published 03 July 1990, (Asano et al) (Col. 1, lines 17-20; claim 4-6, col. 8, lines 34-37)	1-8
X	US, A, 4,983,646 Published 08 January 1991, (Jaouen et al.) (Claim 1, 2, col. 1, lines 20-30)	1-4,6
X	US, A, 4,933,157 Published 12 June 1990, (Counsell et al.) (Col. 7, lines 64-68, col. 2, lines 10-23, claim 5)	1-8
X Y	US, A, 4,541,957 Published 17 September 1985, (Nakatsuka et al.) (Col. 1, lines 5-40) claim 1	1-6 8
<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p>* Special categories of cited documents: †</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 48%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"Δ" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
12 June 1992	29 JUN 1992	
International Searching Authority	Signature of Authorized Officer	
ISA/US	C. Sayala	

